Nanobiotechnology: Nanoscale Chemical Reactions and Separations

This project focuses on the development of nanoscale structures to facilitate the performance of ultra-small volume chemical reactions and separations. The work is associated with the Single Molecule Manipulation and Measurement competence program whose purpose is to study the behavior of biomolecules one molecule at a time to elucidate the differences that make them uniquely beneficial or detrimental. The nanoscale structures that we are designing are composed most often of phospholipid molecules and are self-assembled spherical or tubular structures with diameters ranging from tens to hundreds of nanometers. We have used these liposome structures to facilitate the assembly of structures from other materials. The purpose of this work is to develop nanometer-sized structures that can ultimately be incorporated into microsystems (microfluidics and microelectromechanical systems [MEMS]) for use in studying the behavior of very small numbers of biological molecules with fine control.

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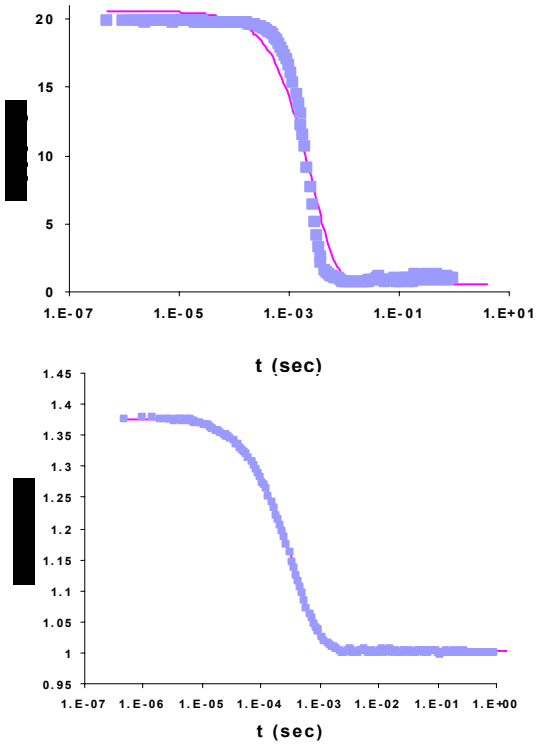
There were two major accomplishments associated with the NIST microfluidics research in 2005:

- Fabrication of microfluidic systems to facilitate the rapid and reproducible formation of liposomes with encapsulated fluorescent molecules
- Using liposomes to template the gelation of macromolecules into nanospheres of controlled size.

Due to their amphiphilic nature, when phospholipid molecules are dispersed in water they self-assemble into bilayer membranes to form structures called liposomes that are often spherical and encapsulate an aqueous internal volume. Liposomes made using bulk techniques range in size from 50 nm to tens of micrometers encapsulating volumes that are measured in attoliters to picoliters. Water-soluble molecules can be readily incorporated into the liposomes upon formation. The ultimate goal of our work is to use liposomes as discrete packages to sequester very small amounts of reagents in order to control finely their reaction. For this purpose, two characteristics of the liposome population are critical – the liposome size and the number of encapsulated molecules contained inside each liposome. Ideally, we would like all liposomes in a given population to be identical with a diameter of approximately 100 nm and containing one encapsulated molecule. However, liposomes prepared from bulk techniques generally exhibit a very large polydispersity with either uncontrolled or unpredictable encapsulation efficiency. For example, liposomes made in our laboratory using established techniques range in size from 70 nm to 200 nm in the same preparation.

Recently, we have demonstrated the automated and controlled formation of liposomes in microfluidic systems. We have optimized the flow conditions and channel dimensions to give optimum performance with respect to monodispersity in size. The graphs below show dynamic light scattering data comparing liposomes prepared with traditional techniques to those formed in a more well controlled microfluidic environment. The autocorrelation function produced by the microfluidically produced liposomes can be more closely matched by a single exponential fit than the liposomes produced by traditional methods,



The top graph shows the dynamic light scattering autocorrelation functions of traditional bulk-scale formed liposomes, and the microfluidic-formed liposomes are shown in the bottom graph. Blue squares represent actual data and the pink line is a single mode exponential fit of the data. The fact that the microfluidic-formed liposomes more closely match a single-mode exponential fit of the data is indicative of their decreased polydispersity

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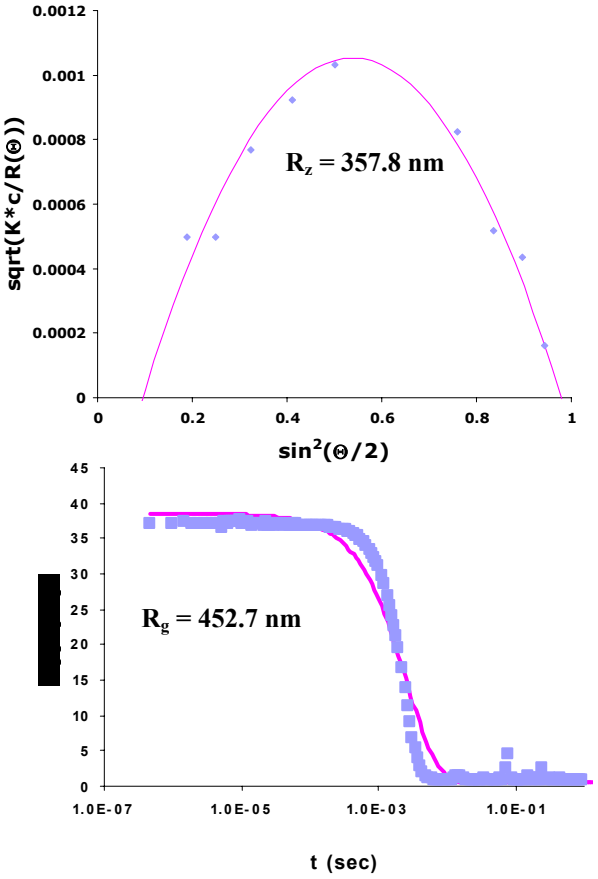
Further, we have used this system to make alginate nanospheres of controlled size by using liposomes to template alginate hydrogel formation. Alginate exists as a monomeric polysaccharide in aqueous buffers, however in the presence of divalent cations it gels to form a polymeric hydrogel. Liposomes can be thermally actuated to have dramatically increased bilayer permeability near the lipid chain melting transition temperature (T_m). Thus, at a controlled temperature, a liposome can be thermally triggerable to allow the transfer of ionic species between the intra- and extravesicular environments. Thus, by encapsulating alginate in liposomes of a controlled size and then introducing the divalent cation calcium to the alginate in the intravesicular space through thermal actuation, the alginate will gel to form a hydrogel nanosphere. After removing the lipsome from the alginate nanosphere by treatment with detergent, the hydrogel diameter closely matches the size of the original liposome.



Impact: The characteristics of fluidic flow in a micrometer-scale channel can be used to precisely control the distribution of chemical conditions and mechanical forces so that they are constant on a length scale equivalent to that of a liposome. Hence, forming liposomes in a micrometer-scale flow field results in more homogenous conditions during liposome self-assembly and resultant liposome populations that are more uniform in size, hence of low polydispersity. These liposomes can then be used to template the formation of hydrogels that might more closely mimic the natural environment of the biological cell.

Future Plans: The work is critical to our efforts in the development of new tools for observing and characterizing single-molecule behavior. The characterization of single biomolecules, rather than the study of ensembles of biomolecules, is an important topic in the field of biology since it has been elucidated that the presence and behavior of the biological outlier or the mutant version of the biomolecule can facilitate amplification of that species resulting in catastrophic consequences as highlighted in recent reports on prions. This year, we have made considerable progress toward packaging single molecules, and performing controlled reactions with a few molecules. Future work will involve further characterization of encapsulated single DNA and RNA molecules so that we can study their behavior one at a time.

Figure:
Multiangle static laser light scattering data (top panel) and dynamic light scattering data (bottom panel) of alginate nanophere hydrogels. Alginate hydrogel size matches the original liposome template size. The fact that the ratio of R_z to R_g is approximately 0.77 is in agreement with theoretical predictions for a solid sphere.